Modulation of mitochondrial adenosine triphosphate–sensitive potassium channels and sodium-hydrogen exchange provide additive protection from severe ischemia-reperfusion injury

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Background: Preconditioning and inhibition of sodium-proton exchange attenuate myocardial ischemia-reperfusion injury by means of independent mechanisms that might act additively when used together. The hypothesis of this study is that treatment with a sodium-proton exchange inhibitor and a mitochondrial adenosine triphosphate–sensitive potassium channel opener produces superior functional recovery and a greater decrease in left ventricular infarct size compared with treatment with either drug alone in a model of severe global ischemia.

Methods: Isolated crystalloid-perfused rat hearts (n = 8 hearts per group) were administered vehicle (control, 0.04% dimethyl sulfoxide), diazoxide (100 μmol/L in 0.04% dimethyl sulfoxide), cariporide (10 μmol/L in 0.04% dimethyl sulfoxide), or diazoxide and cariporide before 40 minutes of ischemia at 35.5°C to 36.5°C and 30 minutes of reperfusion.

Results: The combination group had superior postischemic systolic function compared with that seen in the cariporide, diazoxide, and control groups (recovery of developed pressure: 91% ± 7% vs 26% ± 5%, 35% ± 6%, and 16% ± 3%, respectively; $P < .05$). Postischemic diastolic function in the combination group was superior compared with that seen in the other groups ($change_{pre\text{-}post}$ diastolic pressure of 67 ± 4 mm Hg with control, 49 ± 11 mm Hg with diazoxide, 59 ± 10 mm Hg with cariporide, and 3 ± 3 mm Hg with diazoxide and cariporide combination; $P < .05$). The left ventricular infarct area was less in the combination group compared with that in the cariporide, diazoxide, and control groups (6% ± 2% vs 35% ± 7%, 25% ± 3%, and 37% ± 9%, respectively; $P < .05$).

Conclusions: Combining a selective mitochondrial adenosine triphosphate–sensitive potassium channel opener with a selective reversible inhibitor of sarcolemmal sodium-proton exchange improves recovery of contractile function from severe global ischemia in the isolated buffer-perfused rat heart. The putative mechanism for this benefit is superior protection of mitochondrial function.

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Ischemic preconditioning has been shown to improve cardiac functional recovery after ischemia and reperfusion. One postulated mechanism for this effect is modulation of mitochondrial adenosine triphosphate–sensitive potassium (K^+_{ATP}) channels. Opening of this channel protects mitochondria, and thus the myocyte, from ischemia-reperfusion injury. Discovery of mitochondrial K^+_{ATP} channel–mediated protection from ischemia-reperfusion injury stimulated efforts to reproduce the protective effect of ischemic precon-

ditioning by means of pharmacologic manipulation. Improved postischemic mitochondrial and cardiac function have now been demonstrated after treatment with drugs (eg, diazoxide) that open mitochondrial K_{ATP} channels in isolated hearts,[1-5] isolated myocytes,[6] isolated mitochondria,[7] skinned myocardial bundles,[8] and isolated human atrial trabeculae.[9]

Pharmacologic inhibition of the sarcolemmal Na^+/H^+ exchanger during ischemia and reperfusion is another pharmacologic means to reduce ischemia-reperfusion injury.[10] The benefit of Na^+/H^+ exchange (NHE) inhibition is based on limiting the net increase in intramyocyte Na^+ that results from sarcolemmal NHE during ischemia and reperfusion. Attenuating this acute increase in cytosolic Na^+ in turn decreases the subsequent increase in myocyte Ca^{++} caused by sarcolemmal Na^+/Ca^{++} exchange. NHE inhibition is protective against ischemia-reperfusion injury in the isolated heart,[11-14] in intact pig hearts supported on cardiopulmonary bypass,[15,16] and in a canine infarct model.[17]

The mechanisms for the protective effects of NHE inhibition and pharmacologic preconditioning mediated by mitochondrial K^+_{ATP} channels are independent but have the common end point of mitochondrial protection, which is achieved either directly through opening of the K^+_{ATP} channel or indirectly by sparing the mitochondria from exposure to high cytosolic Ca^{++}. It is therefore possible that combining these 2 methods will have an additive effect that provides superior cardiac protection from ischemia-reperfusion injury. We postulate that this protection is achieved by limiting the flux of Ca^{++} into mitochondria and by stabilizing the mitochondrial proton gradient against changes caused by increased Ca^{++}.

Of note, one prior trial examined a combination of the pharmacologic preconditioning agent diazoxide and the NHE inhibitor cariporide in an isolated rabbit heart model of regional ischemia-reperfusion injury. Cariporide alone and diazoxide plus cariporide decreased infarct size measured as a percentage of the area at risk. However, no protection was seen with diazoxide alone, and the combination of diazoxide and cariporide provided no added benefit over that provided by cariporide alone.[18] The absence of a protective effect for diazoxide in this study, as well as the variability that was present within each experimental group, led us to question whether the absence of an additive effect was a function of the experimental design.

The purpose of the present study was to determine whether combining pretreatment with a sarcolemmal NHE inhibitor and a mitochondrial K^+_{ATP} channel opener could improve functional recovery and decrease the size of left ventricular infarction in an isolated rat heart model of severe global ischemia.

Methods

The investigation conformed to the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Resource Council, and was approved by the UAB Institutional Animal Care and Use Committee.

Isolated Heart Preparation

Rats weighing between 290 and 380 g were heparinized, and then anesthesia was obtained with ketamine-xylazine, both administered intraperitoneally. Hearts were removed, immersed in cold buffer, and quickly cannulated and perfused through the aorta with Krebs-Henseleit buffer, having a composition of NaCl (118 mmol/L), KCl (4.7 mmol/L), $CaCl_2$ (2.5 mmol/L), $MgSO_4$ (1.2 mmol/L), $NaHCO_3$ (25 mmol/L), KH_2PO_4 (1.2 mmol/L), Na ethylenediamine tetra-acetic acid (0.05 mmol/L), and glucose (11 mmol/L) gassed with 95% oxygen and 5% carbon dioxide. A latex balloon was inserted into the left ventricular cavity and anchored in place with sutures. The hearts were immersed in buffer (35.5°C-36.5°C) and paced. Perfusion pressure was maintained at 100 mm Hg by using a custom-built perfusion system that permits perfusion at either constant pressure or constant flow. The system, acting through a stepper motor connected to a peristaltic pump, delivers either a constant flow preset by the operator or provides a flow sufficient to maintain a preset perfusion pressure. A constant-pressure mode was used in this study.

Left Ventricular Pressure Recording

Left ventricular pressure waveforms, coronary flow, and coronary perfusion pressures were measured and recorded on a laboratory microcomputer and stored on the hard drive for later analysis. Software determined diastolic and systolic pressures of the stored data and measured positive and negative dP/dt, coronary vascular resistance, and contracture time and force.

Area of Infarction

At the end of each experiment, hearts were perfused for an additional 2.5 hours; frozen, thawed, and sliced into 2-mm slices; incubated in 1% triphenyl tetrazolium chloride for 20 minutes at 37°C; and fixed in formalin. The slices were pressed between glass plates, and the total left ventricular area and unstained area (infarct) were traced. The tracings were enlarged with a copying machine, and the areas were quantified with a computerized digitizer.

Experimental Protocol

Each of 32 hearts from male rats were randomly assigned to one of 4 groups: a control group (vehicle only, 0.04% dimethyl sulfoxide [DMSO]), a diazoxide group (100 μmol/L diazoxide dissolved in 0.04% DMSO), a cariporide group (10 μmol/L cariporide dissolved in 0.04% DMSO), and a combination group (100 μmol/L diazoxide plus 10 μmol/L cariporide dissolved in 0.04% DMSO). After a period of equilibration, using normal Krebs-Henseleit buffer as a perfusate to ensure stability of the preparation, we made initial recordings of pressure and flow. Hearts that did not have developed pressures of 100 mm Hg or greater by the end of the equilibration period were excluded from the study. End-diastolic pressures ranged from 2 to 10 mm Hg, and developed pressures ranged from 100 to 179 mm Hg. There were no differences

TABLE 1A. Changes in function and coronary vascular resistance as a result of drug treatment before 40 minutes of ischemia

Group	Measurement time		
	Initial	Treat	Final
Developed pressure (mm Hg)			
Control	137.59 ± 6.65	119.64 ± 6.57	21.55 ± 4.09
Cariporide	142.89 ± 5.57	132.55 ± 6.92	34.45 ± 6.05
Diazoxide	141.16 ± 9.07	144.42 ± 3.28	49.44 ± 9.41
Combined	140.00 ± 7.66	141.44 ± 4.37	125.19 ± 7.17
End-diastolic pressure (mm Hg)			
Control	4.40 ± 0.30	1.81 ± 0.43	71.39 ± 4.04
Cariporide	3.41 ± 0.44	1.96 ± 0.52	62.68 ± 9.16
Diazoxide	3.48 ± 0.47	2.06 ± 0.23	52.01 ± 10.25
Combined	3.73 ± 0.47	1.39 ± 0.63	6.98 ± 2.68
Coronary vascular resistance (mm Hg · min^{-1} · mL^{-1})			
Control	5.38 ± 0.32	5.56 ± 0.25	7.54 ± 1.09
Cariporide	5.32 ± 0.34	6.18 ± 0.42	5.94 ± 0.47
Diazoxide	5.25 ± 0.34	3.05 ± 0.08	4.29 ± 1.40
Combined	4.74 ± 0.29	3.04 ± 0.08	4.92 ± 0.24

For each variable measured, longitudinal comparisons were made between the within-group values (initial, treatment, and final), and cross-sectional comparisons were made between groups at each measurement time (Tables 1B-1D). These comparisons were made within the framework of a repeated-measures analysis of variance that contained effects for group, time, and the group-by-time interaction and assumed an unstructured covariance matrix for the within-subject lack of independence. In all cases the group-by-time interaction was found to be statistically significant ($P < .0001$). The comparisons were made between the least-squares means, and the Tukey adjustment for multiple comparisons was used to determine the significance level in all cases. Initial, Before drug; treat, after the drug was started and before ischemia; final, after ischemia.

between groups before treatment. After the pretreatment data acquisition, the perfusate was switched to one of the 4 aforementioned groups, and perfusion was continued for 10 minutes with pacing. At 10 minutes, pacing and perfusion were stopped (30 minutes of ischemia in the pilot study, n = 3 hearts per group; 40 minutes of ischemia in the final study, n = 8 hearts per group), and the hearts were made globally ischemic. Temperature was maintained at 35.5°C to 36.5°C for the entire duration of ischemia.

At reperfusion, perfusion pressures were carefully controlled because excessive perfusion pressures were found to be harmful to postischemic hearts. Perfusion pressure was maintained at 60 mm Hg for the first 15 minutes and then increased in increments of 10 mm Hg every 5 minutes. Pacing at 330 beats/min was resumed when the perfusion pressure reached 100 mm Hg. At 30 minutes of reperfusion, a final recording of cardiac function, coronary flow, and perfusion pressure was made.

Diazoxide was obtained from Sigma Chemical Co (St Louis, Mo). Cariporide was a generous gift from Aventis Pharma Deutschland GmbH (Frankfurt am Main, Germany).

Statistical Analysis

Analysis of stored waveform data included measurements of diastolic and systolic pressures, positive and negative dP/dt, time to peak pressure, diastolic decay time constant (τ), and coronary vascular resistance. Results are shown as means ± SEM.

The statistical design for the variables listed above is typical of a longitudinal model with repeated measurements within an experimental unit over time and with the experimental units in independent groups consisting of a control group and 3 drug treatment groups. The data are balanced in the usual statistical sense, with equal numbers of animals in each group. The data were analyzed by using a fixed-effects repeated-measures analysis of variance, with possible lack of independence in the measurements within a subject being modeled with an unstructured covariance matrix. Note that other possible models for the correlation structure were considered, but the unstructured covariance matrix gave the best fit, as measured with likelihood ratio tests and the Akaike criterion for model identification. The model contained effects for group, time, and a group-by-time interaction. Least-squares means were calculated for the group-by-time interactions, and multiple comparisons were carried out with multicomparison adjustment by using the Tukey method (SAS-PC version 8.00; SAS Institute Inc, Cary, NC).

Infarct area was measured as a percentage of the area at risk. Between-group comparisons of infarct area, time to contracture, and force of contracture were done by using the Duncan multiple range test (SAS-PC version 8.00, SAS Institute Inc).

Results

Initial (Pretreatment) Conditions

Developed pressures for the control, diazoxide, cariporide, and combination groups were 137.6 ± 7.1, 141.2 ± 9.7, 142.9 ± 6.0, and 140.0 ± 8.2 mm Hg, respectively. End-diastolic pressures for these groups were 4.4 ± 0.3, 3.5 ± 0.5, 3.4 ± 0.5, and 3.7 ± 0.5 mm Hg, respectively. There were no significant differences in developed pressures or end-diastolic pressures among these groups. Coronary vascular resistance was likewise similar among groups before ischemia: 5.38 ± 0.34, 5.25 ± 0.36, 5.32 ± 0.37, and 4.74 ± 0.31 mm Hg × min^{-1} × mL^{-1}, respectively (Table 1).

TABLE 1B. Developed pressure within-group comparisons (*P* values)

	Control		Cariporide		Diazoxide		Combined	
	Treat	Final	Treat	Final	Treat	Final	Treat	Final
Initial	.0105	.0001	.049	.0001	.9999	.0001	.9999	.8813
Treat		.0001		.0001		.0001		.1770

	Cariporide	Diazoxide	Combined
Initial time point			
Control	.9999	.9999	.9999
Cariporide		.9999	.9999
Diazoxide			.9999
Treatment time point			
Control	.9635	.0749	.2495
Cariporide		.9124	.9929
Diazoxide			.9999
Final time point			
Control	.8226	.2688	.0001
Cariporide		.9660	.0001
Diazoxide			.0001

See footnote for Table 1A.

TABLE 1C. End-diastolic pressure within-group comparisons (*P* values)

	Control		Cariporide		Diazoxide		Combined	
	Treat	Final	Treat	Final	Treat	Final	Treat	Final
Initial	.0001	.0001	.0001	.0001	.3924	.0027	.1254	.9933
Treat		.0001		.0001		.0016		.5343

	Cariporide	Diazoxide	Combined
Initial time point			
Control	.7811	.8756	.9835
Cariporide		.9999	.9999
Diazoxide			.9999
Treatment time point			
Control	.9999	.9999	.9997
Cariporide		.9999	.9984
Diazoxide			.9058
Final time point			
Control	.9990	.8261	.0001
Cariporide		.9996	.0002
Diazoxide			.0095

See footnote for Table 1A.

Effect of Preischemic Treatment

When comparing the pretreatment with posttreatment developed pressures within groups, developed pressures in the control and cariporide groups decreased significantly, whereas pressures in the diazoxide and combination groups were unchanged (Table 1). There were no differences among groups in end-diastolic pressure after preischemic drug treatment, but within-group comparisons of end-diastolic pressure showed significant decreases in the control and cariporide groups. In both the diazoxide and the combination groups, there were significant decreases in coronary vascular resistance during the preischemic treatment period, with no changes in the control and cariporide groups.

Preliminary Studies With 30 Minutes of Ischemia

Preliminary studies with 30 minutes of global ischemia (n = 3 hearts per group) showed a protective effect of diazoxide and cariporide when used alone but failed to show a statistically significant increment of protection with a combination of the drugs (Figure 1, *A*). The developed pressures in control hearts recovered to 17.6% ± 5.6%, whereas the treated groups had significantly better recovery (diazoxide, 78.5% ± 6.4%; cariporide, 76.4% ± 4.5%; combination,

TABLE 1D. Coronary vascular resistance within-group comparisons

	Control		Cariporide		Diazoxide		Combined	
	Treat	Final	Treat	Final	Treat	Final	Treat	Final
Initial	.9639	.5324	.5387	.9477	.0001	.9999	.0001	.9989
Treat		.6185		.9999		.9988		.0001

	Cariporide	Diazoxide	Combined
Initial time point			
Control	.9999	.9999	.9369
Cariporide		.9999	.9759
Diazoxide			.9905
Treatment time point			
Control	.9739	.0001	.0001
Cariporide		.0001	.0001
Diazoxide			.9999
Final time point			
Control	.9650	.7893	.4754
Cariporide		.9910	.7198
Diazoxide			.9999

See footnote for Table 1A.

91.3% ± 9.3%; $P < .05$ vs control). The differences in recovery of developed pressures between the combination and single-drug groups did not attain statistical significance. End-diastolic pressure in control hearts increased to 57.5 ± 5.1 mm Hg. The increase in diastolic pressure for the combination and single-drug groups was significantly less than that seen in the control group (diazoxide, 4.5 ± 2.7 mm Hg; cariporide, 5.3 ± 2.6 mm Hg; combination, 2.5 ± 2.0 mm Hg; $P < .05$ vs control; Figure 1, *B*). On the basis of these observations, the ischemic interval was increased to 40 minutes for the subsequent experiments (n = 8 hearts per group) to magnify any potential protective effects of combination therapy.

Studies With 40 Minutes of Ischemia

When the ischemic time was lengthened from 30 minutes to 40 minutes, protection by single drug treatment was lost, whereas the combination treatment remained protective (Table 1 and Figures 2 and 3). The percentage of recovery of developed pressure after 40 minutes of global ischemia was significantly better in hearts pretreated with the combination of drugs compared with that seen in control hearts (91.2% ± 6.6% combination vs 15.5% ± 3.2% control, $P < .01$; Figure 2, *A*). Diazoxide as sole therapy provided less recovery of developed pressure (34.9% ± 6.1%, $P = .27$ vs control). Similarly, recovery of developed pressure after cariporide treatment alone was not significantly different from recovery in the control group ($P = .82$ vs control).

Diastolic pressures increased by 67.0 ± 4.1 mm Hg in the control group, 48.5 ± 10.9 mm Hg in the diazoxide group, and 59.3 ± 9.6 mm Hg in the cariporide group (Figure 2, *B*). In the combination group the diastolic pressure increase was only 3.3 ± 3.2 mm Hg ($P < .01$ vs control or single-drug therapy).

Infarct areas, expressed as a percentage of the total left ventricular area (Figure 3), were similar for the control group (37.3% ± 9.1%), the diazoxide group (24.8% ± 2.6%), and the cariporide group (34.7% ± 7.1%). In contrast, the infarct area was less in the combination group (5.8% ± 1.8%, $P < .01$ vs control and single-drug therapy).

During ischemia, contracture developed in all hearts. The diazoxide-treated hearts had the longest contracture time (21.5 ± 1.3 minutes, $P = .011$) compared with that seen in the other groups. The times to contracture for the control and single-drug therapy groups ranged from 16.1 to 17.6 minutes and were not significantly different from each other (Table 2). The force of contracture was greatest in the cariporide and control groups (38.0 ± 2.4 and 35.4 ± 5.3 mm Hg, respectively) and was different from that seen in the diazoxide and combination groups (24.5 ± 1.7 and 20.3 ± 1.7 mm Hg, respectively; $P < .01$ vs control and cariporide groups).

Times to peak developed pressure during systole were no different among groups before ischemia (range, 63.1-67.4 ms). These times lengthened after ischemia in all groups (range, 67.2-75.3 ms), but the increases were insignificantly different from each other.

The diastolic relaxation time constants (τ) were similar among groups before ischemia. Thirty minutes after reperfusion, they were significantly longer in the control group than in each of the other treatment groups (Table 2). Although the time constant was shortest in the combination group, it was not statistically different from that seen in the other treatment groups.

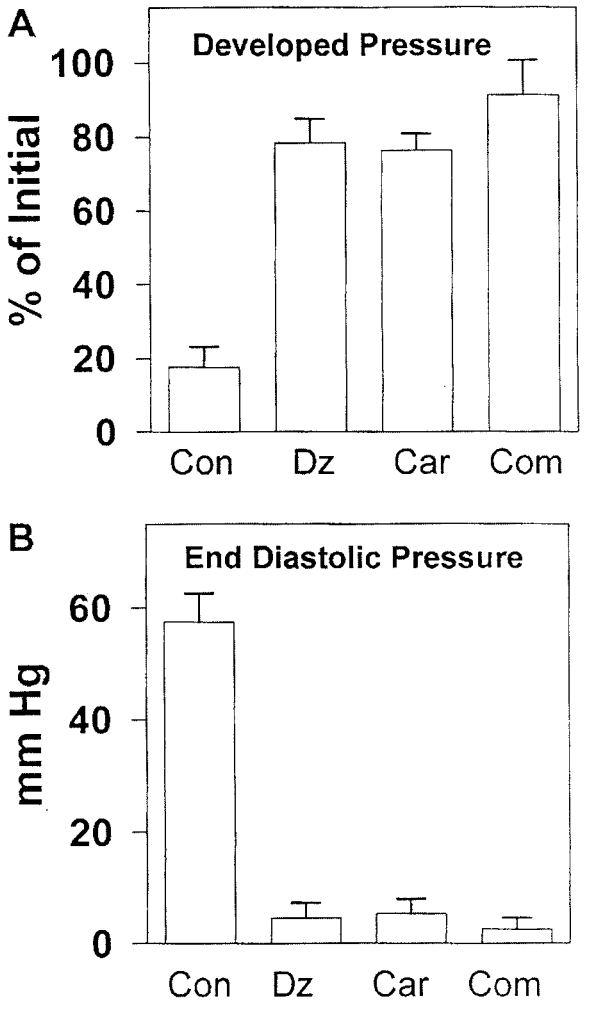

Figure 1. A, Postischemic developed pressure as a percentage of preischemic developed pressure for 30 minutes of ischemia. B, Postischemic left ventricular end-diastolic pressure (in millimeters of mercury) for 30 minutes of ischemia. *Con,* Control; *DZ,* diazoxide; *Car,* cariporide; *Com,* combination treatment.

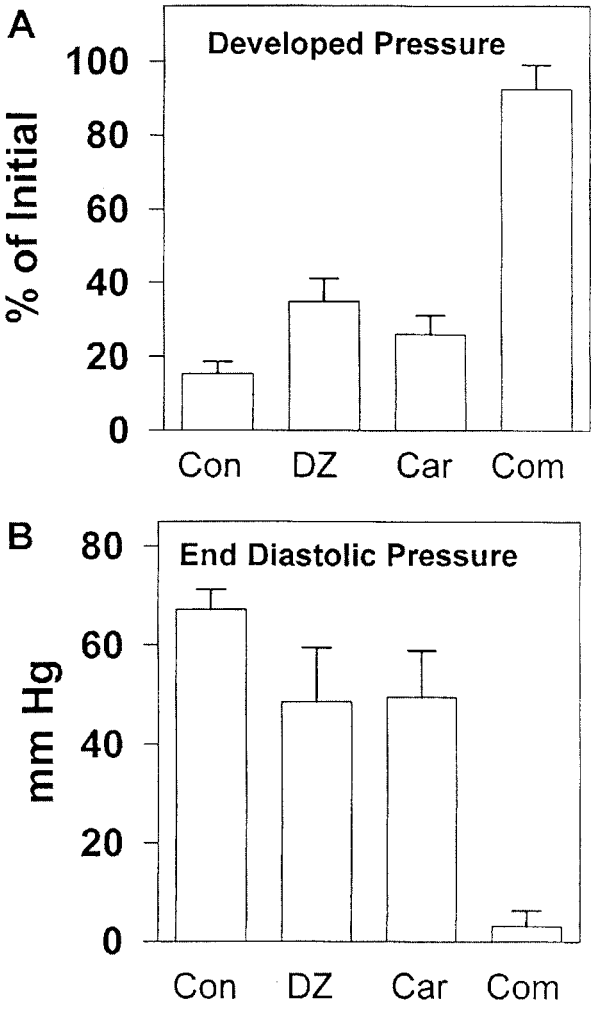

Figure 2. A, Postischemic developed pressure as a percentage of preischemic developed pressure for 40 minutes of ischemia. B, Postischemic left ventricular end-diastolic pressure (in millimeters of mercury) for 40 minutes of ischemia. *Con,* Control; *DZ,* diazoxide; *Car,* cariporide; *Com,* combination treatment.

After 30 minutes of reperfusion, the diazoxide group had the lowest coronary vascular resistance compared with that seen in the control group, although this comparison did not attain significance ($P = .79$). The other groups were statistically similar to the control group.

Discussion

These experiments show that in a Langendorff crystalloid-perfused isolated rat heart model the combination of preischemic treatment with a sarcolemmal NHE inhibitor and a mitochondrial K^+_{ATP} channel opener additively improves systolic and diastolic functional recovery and limits infarct area induced by 40 minutes of global ischemia. In the preliminary study we observed that a combination of diazoxide and cariporide provided an improvement that did not attain statistical significance over each drug used alone as protection from 30 minutes of normothermic ischemia. When the ischemic interval was extended to 40 minutes for the subsequent experiments, the protection provided by diazoxide treatment was reduced, and the protection provided by cariporide was essentially lost. However, the combination of diazoxide and cariporide afforded protection. Postischemic diastolic pressures remained low after 30 minutes of ischemia in the single-drug treatment groups but increased significantly after 40 minutes of ischemia. Combination treatment prevented an increase in postischemic diastolic pressure.

The additive beneficial effects of diazoxide and cariporide on systolic and diastolic function confirm that the

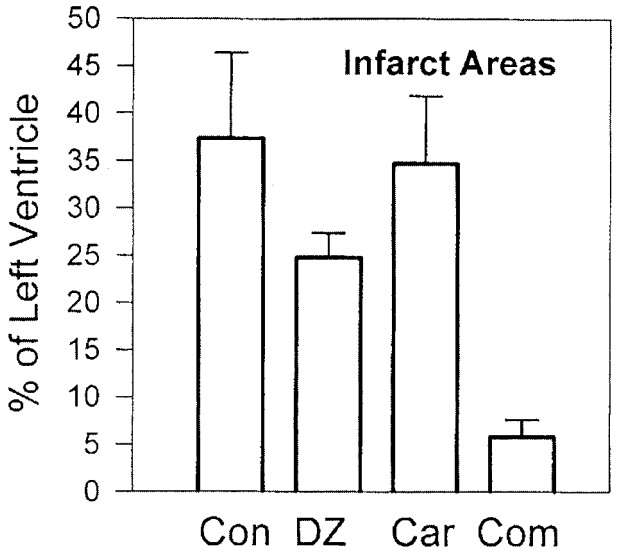

Figure 3. Infarct area as a percentage of left ventricular area (ie, area at risk in global ischemia) for 40 minutes of ischemia. *Con*, Control; *DZ*, diazoxide; *Car*, cariporide; *Com*, combination treatment.

mechanisms for protection by NHE inhibition and mitochondrial K^+_{ATP} channel opening are independent and suggest that they might have a common final effector. Diazoxide opens the mitochondrial K^+_{ATP} channel, thereby partially depolarizing the mitochondrial membrane potential. This is thought to cause a decrease in the amount of voltage-driven calcium uptake by injured mitochondria.[19-22] Increases in mitochondrial calcium are injurious to mitochondria, although the precise mechanism for calcium-mediated mitochondrial injury has not been fully defined. Defining this mechanism at a molecular level in the mitochondria is a long-range goal of this laboratory.

The highly selective NHE inhibitor cariporide limits sodium uptake during ischemia and reperfusion, thereby preventing excessive cytoplasmic calcium uptake through the sarcolemmal sodium-calcium exchanger.[23,24] We hypothesize that diazoxide and cariporide can act additively to improve myocyte ion homeostasis, thereby protecting mitochondria from calcium-mediated injury. It is known that an increase in cytoplasmic calcium can cause myofibrillar hypercontracture,[25] activation of cytoplasmic proteolytic enzymes,[26-28] and mitochondrial damage.[29]

In this study the test drugs were given before ischemia, and this might not always be possible in clinical surgery. However, cariporide and diazoxide still have an effect in controlling calcium uptake when given only during reperfusion.[8,16] Thus this limitation diminishes but does not eliminate the usefulness of combination therapy in situations in which ischemia starts before the drugs can be given. The

TABLE 2. Ischemic contracture variables and postischemic relaxation time constants

Group	Contracture time (min)	Contracture amount (mm Hg)	Change in τ (ms)
Control	16.8 ± 1.6	35.4 ± 5.3	410 ± 108
Diazoxide	21.5 ± 1.3*	24.5 ± 2.0†	126 ± 55†
Cariporide	16.1 ± 0.9	38.0 ± 2.4	111 ± 35†
Combination	17.6 ± 0.8	20.3 ± 1.7*	−3.4 ± 2.3†

All values are given as means ± SEM.
*$P < .05$ and †$P < .01$ compared with the control group (n = 8 hearts per group).

asanguineous perfusate used in this study is another limitation because it does not perfectly mimic the clinical situation, in which leukocytes contribute to reperfusion injury. Parallel studies in an intact porcine model are underway in this laboratory to validate the findings from the rat studies.

Relaxation time constants, which are an index of active diastolic relaxation,[30] were significantly increased in the control group after ischemia ($P < .01$), marginally increased in the diazoxide group ($P = .056$), increased in the cariporide group ($P = .027$), and unchanged in the combination treatment group ($P = .186$). Impairment of diastolic relaxation might be caused by ischemic injury to the sarcoplasmic reticulum. This injury is presumably most severe in the case of the control hearts and results in decreased calcium uptake rates during diastole. The combination of cariporide and diazoxide might improve protection of the sarcoplasmic reticulum compared with protection provided by each drug alone.

Infarct areas were measured in the hearts with 40 minutes of ischemia. The global ischemic injury produced patchy areas of necrosis in the control hearts, as well as in the diazoxide- and cariporide-treated hearts. These areas of infarction were significantly decreased in size when diazoxide and cariporide were combined as preischemic treatment.

The combination of diazoxide and cariporide was previously examined in an intact rabbit model of regional ischemia and infarction, but no significant reduction in infarct size was observed.[18] The reason for the discrepancy between our results and these other results is not clear but could be due to the difference in species, to the fact that the intact rabbit hearts were blood perfused while our hearts were buffer perfused, or to the effective final concentration of agents at the myocyte level. It has been observed that diazoxide binds immediately to plasma proteins, thus decreasing the effective concentration.[31] Our study indicates that an additive beneficial effect exists and justifies further investigation of combination therapy, including dose-response studies and experiments to confirm the putative mechanisms for its benefit.

Another issue is the effect of changes in coronary vascular resistance and flow on recovery of cardiac function.

The diazoxide group had significantly lower coronary vascular resistance than the cariporide or control groups, but recovery of systolic and diastolic function in the diazoxide group was significantly less than in the combination group. Thus the improved functional recovery we observed in the combination-treated hearts was not solely due to an increase in coronary flow during reperfusion.

The concept of combining NHE inhibition with ischemic or pharmacologic preconditioning to improve recovery from ischemia is not new, although the mechanisms for an additive benefit have not been determined, and methods for clinical use of this information have not been developed. Other combinations that have been studied include ischemic preconditioning and the NHE1 inhibitor BIIB 513,[24] ischemic preconditioning and ethylisopropylamiloride (another NHE inhibitor),[32,33] and ischemic preconditioning and cariporide.[1] Sato and colleagues[34] combined adenosine and diazoxide in a cellular model of simulated ischemia. In each of these studies, recovery of function was improved, and infarct area was reduced or cell survival was increased when the combination was used.

The present study indicates that pharmacologic preconditioning with a selective mitochondrial K^+_{ATP} channel opener (diazoxide) acts additively with NHE inhibition to provide protection from severe ischemia-reperfusion injury. Thus our findings, if confirmed in studies using more clinically relevant models, will support the use of drugs that are in preclinical or clinical development (eg, nicorandil [a sarcolemmal and mitochondrial K^+_{ATP} channel opener] and cariporide) as combination therapy to treat ischemia-reperfusion injury in cardiac surgery. Moreover, the findings suggest that the mechanism for this additive protection is improved protection of myocyte ion homeostasis and mitochondrial function during ischemia and reperfusion. This putative mechanism provides the basis for further development of novel methods for mitochondrial and myocardial protection that might, in the future, be useful for treating severe ischemia-reperfusion injury in cardiac surgery.

In summary, we have shown that combining a selective mitochondrial K^+_{ATP} channel opener with a selective reversible inhibitor of sarcolemmal NHE decreases infarct size and improves recovery of systolic and diastolic contractile function after severe global ischemia in the isolated buffer-perfused rat heart. We hypothesize that the simultaneous application of these 2 drugs before ischemia not only limits the total gain in cytoplasmic calcium with ischemia and reperfusion but also limits the gain in calcium by the mitochondria, thereby improving mitochondrial function and thus enhancing energy production.

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